Supporting Information

Concerted Complex Assembly and GTPase Activation in the Chloroplast Signal Recognition Particle

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Supporting Information Tables

Table S1. Summary of different classes of mutational effects. Mutations of homologous residues in cpSRP54 and cpFtsY are presented in the same row, except for cpSRP54(Q109A).

Mutational Effect	cpFtsY Mutants	cpSRP54 Mutants	Location
I – Complex formation defective	G288W[1]	G255W	NG interface
II – GTPase activation defective	A168W	A142W	IBD loop
III – Both steps defective	N135A		Dimer interface
	N135W	Q109W	Dimer interface
	D163A	D137A	IBD loop
	R166A	R140A	IBD loop
	A169L	A143L	IBD loop
	A169W	A143W	IBD loop
		G256W	NG interface
Neutral Mutations	F165A		IBD loop
	A167W		IBD loop
	R220A	R193W	Dimer interface
	G288A		NG interface
	G289A		NG interface
	G289W		NG interface
		Q109A	Dimer interface

[1] kinetic parameters from Jaru-Ampornpan *et al* (*1*).

Table S2. Kinetic parameters of additional mutants outside of the IBD loop

Mutants	k_{cat} (min^{-1})		K_M (μM)		k_{cat}/K_M ($\mu M^{-1}min^{-1}$)	
WT	27 ± 3	(1)	1.6 ± 0.3	(1)	18 ± 3	(1)
<u>cpFtsY</u>						
N135A	5.9 ± 2.1	(0.2)	4.3 ± 0.9	(3)	1.4 ± 0.2	(0.1)
N135W	8.0 ± 1.2	(0.3)	2.7 ± 0.9	(2)	3.3 ± 1.2	(0.2)
R220A	26	(1)	2.2	(1)	12	(0.6)
G288A	23	(0.9)	1.1	(0.7)	22	(1)
G289A	24 ± 1	(0.9)	1.1 ± 0.2	(0.7)	23 ± 3	(1)
G289W	29 ± 9	(1)	2.5 ± 1.2	(2)	14 ± 10	(0.8)
<u>cpSRP54</u>						
Q109A	16	(0.6)	2.0	(1)	8.2	(0.5)
Q109W	7.7	(0.3)	4.2	(3)	1.9	(0.1)
R193W	18	(0.7)	2.8	(2)	6.3	(0.4)
G255W[1]	38 ± 3	(1)	41 ± 4	(26)	0.93 ± 0.15	(0.05)
G256W[1]	6.5 ± 0.2	(0.2)	33 ± 6	(21)	0.20 ± 0.03	(0.01)

[1] k_{cat} and K_m values for mutants that are severely compromised in complex formation are determined by extrapolation, as saturation of reaction was not reached up to 50 μM of mutant protein.

Table S3. Mant-GTP binding affinity to the individual mutant GTPases. [1]

GTPase constructs	K_d (μM)
cpFtsY	
WT	1.8
D163A	1.2
R166A	3.1
A168W	3.1
A169L	ND
A169W	3.3
G288W	0.92
cpSRP54	
WT	6.7
D137A	2.6
R140A	58
A142W	2.6
A143L	68
A143W	3.5
G255W	8.2
G256W	35

[1] Mant-GTP (Jena Bioscience) binding assay was performed as described in Jaru-Ampornpan *et al* (*2*).

Table S4. Basal GTPase activity of cpFtsY mutants

cpFtsY construct	k_{cat} (min^{-1})	K_m (μM)
WT	0.0039 ± 0.0009	1.1 ± 0.4
N135A	0.0077 ± 0.0022	0.34 ± 0.04
N135W	0.0020 ± 0.0002	1.3 ±1.1
R166A	0.0028 ± 0.0003	1.9 ± 0.1
D163A	0.011	0.44
F165A	0.0124 ± 0.0096	1.2 ± 0.3
A167W	0.0032	1.9
A168W	0.0023 ± 0.0002	1.1 ± 0.6
A169L	0.0038	2.1
A169W	0.0088 ± 0.0029	1.9 ± 0.9
R220A	0.0050 ± 0.0008	1.6 ± 0.3
G288A	0.0031 ± 0.0002	0.58 ± 0.18
G288W	0.0039 ± 0.0002	0.34 ± 0.16
G289A	0.0029 ± 0.0006	0.71 ± 0.41
G289W	0.0030 ± 0.0006	0.40 ± 0.25

Supporting Information Figures

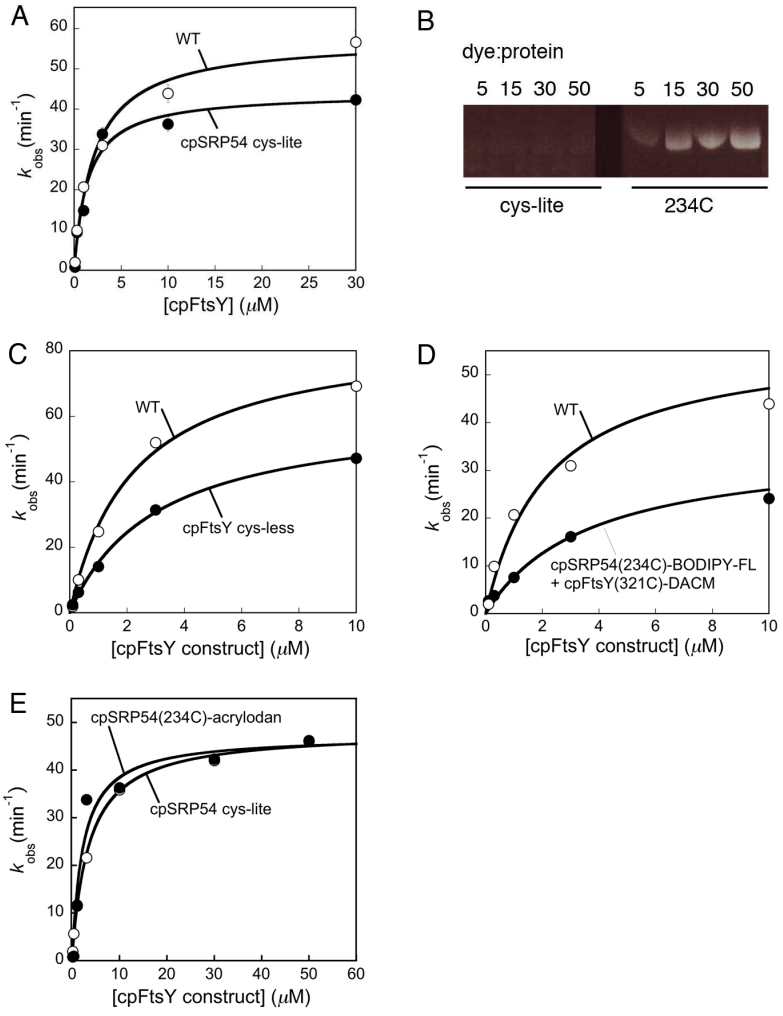

Figure S1: cys-lite cpSRP54 and cys-less cpFtsY and their fluorescently labeled versions are enzymatically active. All GTPase assays were performed as described in the *Methods*,. cpSRP54(234C) refers to the cys-lite construct with a cysteine mutation at position 234 on cpSRP54. cpFtsY(321C) refers to the cys-less construct with a cysteine mutation at position 321 on cpFtsY. (A) The reciprocally stimulated GTPase reaction of cpSRP54 WT (○) or cpSRP54 cys-lite (●) with cpFtsY. Fits of the data to Eq 5 gave k_{cat} values of 56 and 54 min^{-1}, respectively. (B) cpSRP54 cys-lite and cpSRP54(C234) were labeled with BODIPY-FL for 2hrs and loaded on a 10% SDS-PAGE gel. (C) The

stimulated GTPase reaction of cpSRP54 with cpFtsY (○) or cys-less cpFtsY (●). Fits of the data to Eq 5 gave k_{cat} values of 56 and 39 min^{-1}, respectively. (D) The stimulated GTPase reaction of cpFtsY with cpSRP54 (○), or of cpFtsY(321C)-DACM with cpSRP54(234C)-BODIPY-FL (●). Fits of the data to Eq 5 gave k_{cat} values of 39 and 20 min^{-1}, respectively. (E) The reciprocally stimulated GTPase reaction of cys-lite cpSRP54 (○) or cpSRP54(C234)-acrylodan (●) with cpFtsY, which gave k_{cat} values of 51 and 43 min^{-1}, respectively.

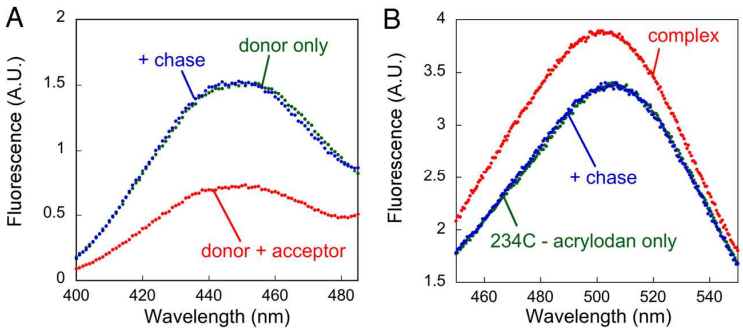

Figure S2: FRET and acrylodan fluorescence signals from cpSRP54•cpFtsY complex could be competed away by EDTA or unlabeled cpSRP54. (A) Fluorescence emission spectra of cpFtsY(321C)-DACM (0.5 μM) in the absence (green) and presence of 2 μM cpSRP54(234C)-BODIPY-FL, with (blue) or without (red) 10 mM EDTA as the chase. Complex assembly was carried out in 2 mM GTP. (B) Acrylodan fluorescence increase upon formation of the cpSRP54•cpFtsY complex (green vs. red) could be chased by a 20-fold excess of unlabeled cpSRP54 (blue). Complex formation was carried out using 0.5 μM cpSRP54(234C)-acrylodan, 2 μM cpFtsY, and 2 mM GTP

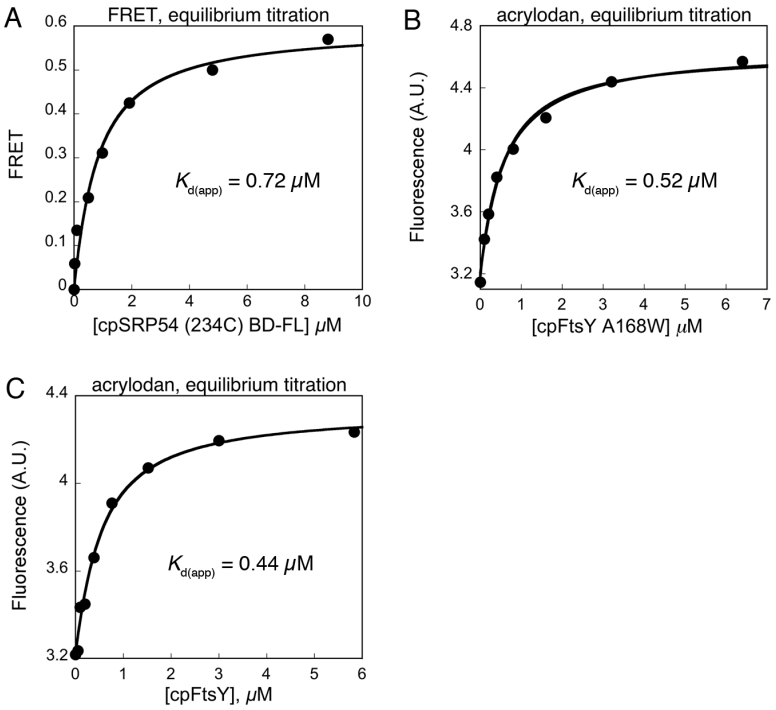

Figure S3: Equilibrium titrations of the cpSRP54•cpFtsY complex in GTP using different fluorescence assays. (A) Complex assembly measured by FRET, using wildtype cpSRP54 and cpFtsY. (B) Complex assembly measured using cpSRP54(234C)-acrylodan and cpFtsY(A168W). (C) Complex assembly measured using cpSRP54(234C, A142W)-acrylodan and cpFtsY. The data were fit to Eqs. 1 or 2 in the Methods, and the K_d values are reported in Table 1.

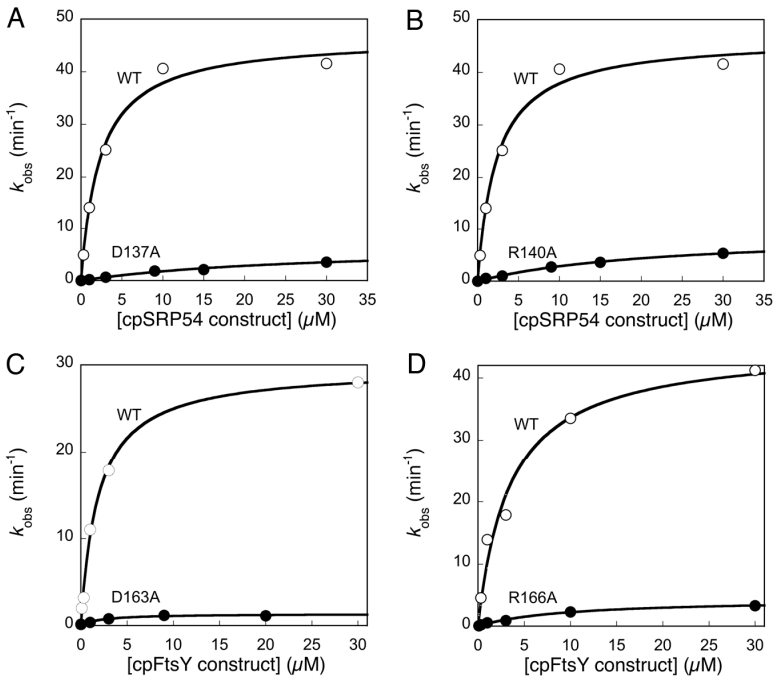

Figure S4: Defects of additional cpSRP54 and cpFtsY IBD loop mutants in complex formation and GTPase activation, measured by the stimulated GTPase reaction. (A, B) The stimulated GTPase reactions of wildtype cpSRP54 (○), and mutants cpSRP54(D137A) (part A, ●) and cpSRP54(R140A) (part B, ●). (C, D) The stimulated GTPase reactions of wildtype cpFtsY (○), and mutants cpFtsY(D163A) (part C, ●) and cpFtsY(R166A) (part D, ●). The figures show representative data, and Table 1 summarizes the average values from two or more measurements.

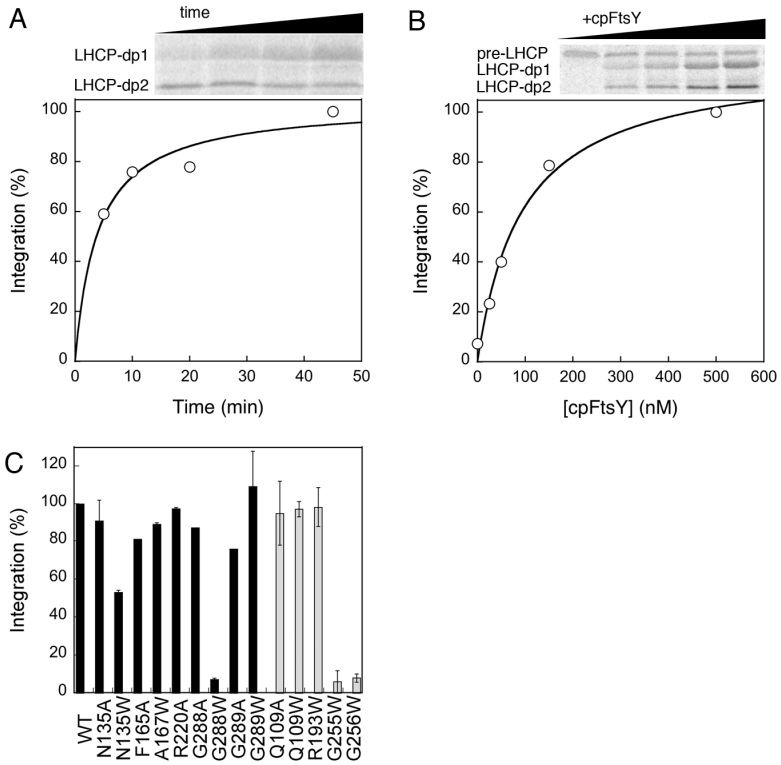

Figure S5: The effects of additional cpSRP54 and cpFtsY mutations on LHCP targeting and integration. (A,B) Time- (part A) and cpFtsY concentration- (part B) dependent targeting and translocation of LHCP, carried out as described in *Methods*. (C) Integration efficiencies of additional cpFtsY (black bars) and cpSRP54 (grey bars) mutants.

REFERENCES

1. Jaru-Ampornpan, P., Nguyen, T.X., and Shan, S. (2009) A distinct mechanism to achieve efficient SRP-SRP receptor interaction by the chloroplast SRP, *Mol. Biol. Cell 20*, 3965-3973.
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3. Zhang, X., Kung, S., and Shan, S. (2008) Demonstration of a two-step mechanism for assembly of the SRP-SRP receptor complex: implications for the catalytic role of SRP RNA, *J. Mol. Biol. 381* 581-593.